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RESICstance Is Futile—But Not in Glioblastoma

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Genomic alterations that occur early in tumorigenesis represent fundamental diver lesions and are perhaps of highest priority as a means to intervene therapeutically. In this issue of *Cancer Cell*, Ozawa and colleagues apply an algorithm to identify early events in glioblastoma and validate their findings in a rigorous manner.

Human tumors, especially high grade malignant neoplasms, exhibit large numbers of genomic changes compared to non-neoplastic cells, but only a subset of those abnormalities are likely to be crucial driver events for tumor initiation and progression. Therefore, an important question when faced with a wealth of genomic data is to identify the critical abnormalities. Early events maintained in the tumor would be presumed to be critical for the tumor and could potentially be identified via serial sampling of tumors over time. However, absent the ability to evaluate multiple samples from the same individual (for example matched primary-metastatic or primary-recurrent pairs), efforts have focused on cross-sectional data with a single time point from multiple individuals with the goal of inferring the likely sequence of genetic events.

The concept of molecular subtypes of glioblastoma (GBM) has a long history, beginning with the distinction of primary (also called de novo) and secondary GBM (Scherer, 1940). Since then, our understanding of GBM subtypes has evolved following the advent of genome-wide studies (Phillips et al., 2006; Verhaak et al., 2010). Transcriptomal studies have led to several classification systems, but a consensus point to two principal and recognized subtypes, the proneural and mesenchymal classes. Further refinement of the proneural class has distinguished tumors with the glioma-CpG island methylator phenotype (GCIMP, often associated with *IDH1* mutation; Noushmehr et al., 2010) from IDH-wild-type/GCIMP-negative tumors. While gene expression studies have led to the concept of GBM subclasses, the collective accumulation of transcriptomal data, and most recently, the study examining gene expression of single cells (Patel et al., 2014), suggest that the distinction between classes may not be so rigid and can also lead to mosaicism and/or the potential for class switching, possibly under the influence of the tumor microenvironment (Bhat et al., 2013). Plasticity among GBM subtypes could then lead to a concept that, while patient GBM samples exhibit transcriptomal diversity, early genetic events may be common to the majority of GBM tumors. To examine this possibility, in this issue of *Cancer Cell*, Ozawa et al. (2014) apply a computational method, Retracing Evolutionary Steps in Cancer (RESIC), in an innovative manner to infer a temporal sequence of genetic alterations that occur during tumorigenesis with reference to transcriptomal subtypes.

In a prior publication, this group applied RESIC to genomic data from colorectal tumors to predict the temporal relationships among alterations in the APC, KRAS, and TP53 genes (Attolini et al., 2010). Briefly, genetic events present in the tumor population are provided as input, and RESIC is designed to return the likely sequence of events that occurred, assuming a monoclonal origin. Genetic alterations deemed to be phenocopies of each other (i.e., independent alterations that similarly impact a specific signaling pathway), are combined into single alteration events. Following this, genetic events that are found to be significantly correlated with each other are identified. The RESIC algorithm then infers the most likely sequence of events among genetic alterations that occur together, and a likely temporal sequence leading up to the specific cancer subtype is constructed. Since this methodology is applicable to large genomic data sets, the authors applied RESIC to GBM samples obtained from The Cancer Genome Atlas data set. As the modern definition of primary GBM has evolved into a group of tumors defined as those without IDH mutation, the authors very intelligently chose to focus only on GCIMP-negative tumors in their analysis. In a previous publication (Cheng et al., 2012), only the focal genetic/genomic changes and later events to highlight distinctions between transcriptomal subtypes were focused upon. Ozawa et al. (2014) extend these findings substantially with a more in-depth analysis, including whole-arm and whole-chromosomal events within the RESIC algorithm to estimate the temporal sequence of events that lead to primary GBM. To validate their approach, predictions were experimentally tested both in culture and, drawing upon the team's strengths, in mouse modeling.

Ozawa et al. (2014) found that whole-chromosome gains of seven and losses of ten are seen in the vast majority (80%–90%) of CIMP-negative GBMs, raising the question: which are the driver genes subsumed by these large genomic changes? With respect to chromosome 7, the authors first correlate the presence of chromosome 7 gain with the expression of genes located on this chromosome to narrow the list of candidate genes to those most tightly regulated to gene dosage. Interestingly, *EGFR*, perhaps the most studied chromosome 7 gene in GBM, was not among those highly correlated to low-level chromosome 7 gain. Subsequently, they identified those chromosome 7 genes and their relevant downstream pathways, which

were correlated to patient outcome. *PDGFA* was found to be the highest ranked gene using these criteria, linking it as a potential early driver event. A similar approach toward the identification of underexpressed genes led to the identification of *PTEN* as a potential chromosome 10 driver tumor-suppressive gene.

With *PDGFA* and *PTEN* identified as driver chromosome 7 and 10 GBM genes, respectively, the authors directly tested these using their RCAS glioma modeling system. Using Nestin/tv-a or GFAP/tv-a mice, they found that overexpression of PDGFA alone was sufficient to initiate gliomas in vivo. While Pten loss alone did not initiate glioma, the addition of *Pten* knockdown to PDGFA overexpression increased malignancy grade and decreased the survival of the mice. Hints toward the relationships between genomic events to transcriptomal subclass were found by a comparison of PDGFA- versus PDGFB-induced tumors, where PDGFA tumors were proneural while PDGFB tumors, accentuated by atypical vasculature and stroma, exhibited a mesenchymal phenotype. Knockdown of *Tp53* in PDGFA-driven glioma led to high-grade histology and shortened mouse survival, while tumors retained their proneural character. To link genetic events and transcriptomal subtype, the authors noted prior work correlating loss of *NF1* with the mesenchymal phenotype (Verhaak et al., 2010) and showed that *Nf1* knockdown in PDGFRA-amplified proneural glioma lead to mesenchymal characteristics. This finding adds to prior evidence to substantiate the hypothesis that the mesenchymal phenotype can arise in the setting of a proneural tumor (Phillips et al., 2006; Bhat et al., 2013). Interestingly, the mechanisms by which this occurred could be related to reports on master transcriptional regulators of the mesenchymal phenotype, including C/EBPβ and RUNX1 (Carro et al., 2010). In vivo, loss of *Nf1* correlated with acquisition of mesenchymal markers (CD44, pSTAT3, and C/EBPβ) in the mouse glioma model.

Overall, using mathematical modeling of human GBM to predict chromosome 7 gain and 10 loss as early genetic events, the authors identified the driver genes underlying these events and validated their role in gliomagenesis in animal models. Based on the finding that the early events lead to proneural GBM, they conclude, consistent with the finding that *NF1* loss is a later event in human GBMs, that mesenchymal GBMs evolve from a proneural-like precursor. While the dynamic nature of gene expression has perhaps contributed to variability in the specifics of GBM transcriptomal classes (Phillips et al., 2006; Verhaak et al., 2010), the fact that the frequency of chromosome 7 gain/10 loss is a general feature of IDH-wild-type/non-CIMP GBM provides a firm basis for focusing on these changes as fundamental to this tumor entity. While EGFR exhibits high-level amplification in a subset of GBM, evidence here suggests this may be a later event. While RESIC is not a discovery tool per se, and is of necessity limited to available genomic data as the input, this work, by combining mathematics with experimental validation, highlights its utility to identify key driver events in GBM.

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